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Life Sciences Group

International Journal of Aquaculture and Fishery Sciences

DOI http://doi.org/10.17352/2455-8400.000038

ISSN: 2455-8400 CC By

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Received: 15 June, 2018
Accepted: 04 July, 2018
Published: 05 July, 2018

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Keywords: Cyprinus carpio; *Abutilon indicum*; Survival Mortality; Haematology; RBC; WBC

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Research Article

Toxicity of Cadmium and their effect on some Heamatological parameters of common carp (*Cyprinus Carpio*) exposed to crude leaf extract of *Abutilon Indicum*

Abstract

The present work aimed to estimate the toxicity of cadmium and their common carp Cyprinus carpio fish, as well as the effect of different concentrations of Cadmium and some haematological parameters. such as, the red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb) Survival mortality was observed in the blood of Common carp Cyprinus carpio treated with Abutilon indicum extract and the result showed that the RBC counts, WBC counts, Hb, Survival mortality were much increased when compared to the control. 8, 16, 24 and 32 of RBC, WBC, and haemoglobin, Survival mortality levels were P<0.05> significantly elevated in the experimental fish over the control and the WBC level was decreased significantly P<0.05> in experimental fish.

Introduction

The aquaculture sector has been extending at an average compounded rate of 9.2% per year since 1970, compared with only 1.4% for seizure fisheries and 2.8% for terrestrial farmed dinner performance systems. With the cursive intensification and commercialization of aquaculture product, contagious diseases are a major proposition suit heavy damage to the fish let the farming industry [1]. Heavy metals are important environmental pollutants and many of them are venomous even at very blazer concentrations. Pollution of the biosphere with poisonous bullion has speeded up theatrically since the inception of the industrial revolution [2]. Heavy ore impurity is known to be the reason of various diseases globally, such as the minamata disease (living mercury poisoning), iItai-itai disease (cadmium pest), arsenous acrimonious poisoning, and airpollution- narrated asthma [3]. Cadmium is a by-product of the mining and smelting of lead and galvanizes and is contain "Top 20 list." It is interested in nickel-cadmium batteries, PVC plastics, and paint pigments. It can be found in soil forasmuch as insecticides, fungicides, slush, and commercial fertilizers that use cadmium in agriculture. Cadmium explains found in reservoirs hold mollusc. Cigarettes also contain cadmium. Lesser-assumed spring of exposure is dental allay, electroplating, engine oil and education. Inhalation narration for 15-50% of absorption through the respiratory system; 2-7% of ingested cadmium is deep in the gastro enteric system. Target organs are the liver, afterbirth; kidneys, breather, and brain steal [4]. Bioaccumulation is the neap of contaminants by variety in concentrations that are the management of importance higher than in the encompassment surrounding. Bioaccumulation is the sum of two signs of progress: bioconcentration and biomagnification. Bioconcentration is the straightforward apprehension of resource by a running system from the ordinary (e.g., irrigate) via skin, gills or lights, whereas biomagnifications event occurs from dietary uptake. Fish that energetically filter out the large totality of hydraulic through their gills are liable to a much higher bio concentration. Additionally, bio magnifications charm place in plundering organisms. The burdened regulus of the booty is transferred to the predator. Water born bullion may change the physiologic and biochemical parameters in Pisces exasperate and cinenchyma. The reaction and survival of aquatic animals depend not only on the biologic state of the animals but also on the poisonousness, with toxicity and semblance and era of exposure to the toxicant [5]. Hematological and biochemical outline in fish is shown to be a sensible demonstrator for the evaluation of drop in a line metabolism under antacid significance. Hence the present study has been carried out the haematological parameters of carp (*Cyprinus carpio*) exposed to crude leaf extract of *Abutilon indicum.*

Materials and Methods

A Live fish (12± 1g) were collected from High-tech fish farm, Madurai, Tamil Nadu, India. The fishes were maintained in

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non-chlorinated water in 20 days. The ground nut oil cake, fish meal and rice bran, tapioca, soybean, were mixeds and sterile condition and mixed to a multivitamin tablet and different concentrations of *Abutilon indicum* extract used for experimental fishes and without plant extract diet for control fish. The food was made into small pellets (Tables 1,2).

Survival and mortality

The survival and mortality rate was calculated by dividing the number of fish died to the total number of fish.

$$\text{Survival rate} = \frac{\text{Number of fish that died}}{\text{Total number of fish}}$$

Haemoglobin content

Shali's Acid haematin method: Fill the graduated tube was filled to the 20 mark (on % scale) with 0.1N HCl. Draw blood by using haemoglobin pipette to 0.02mL. Wipe the tip of the pipette with cotton, so that no blood is left to stick to its outside. Expel blood into the Shali tube containing the HCl solution. Suck a small amount of an acid into the pipette and expel it again into the tube, repeat this twice. Mix the content quickly but gently with glass-rod for 10 min. Add HCI drop by drop, mixing between each addition until the color matches with the standard. Read the amount of solution in the graduated tube, the calibrations give the Haemoglobin concentration [6].

Principle: Hemoglobin can undergo several reactions; it binds with oxygen and carbon monoxide to from oxy hemoglobin and carboxy hemoglobin, respectively. Oxidation of the fesrrous ion to the ferric form results in the formation of methemoglobin. Methemoglobin binds cyanide ions to form cyanmethaemoglobin. Hemoglobin can be measured in any of these forms, but the most satisfactory method of assay from the view point of accuracy and simplicity involves the conversion of all forms of blood hemoglobin to cyanmethaemoglobin.

Hemoglobin + K3 Fe (CN) → 6 Methemoglobin

Methemoglobin + KCN → Cyanmethemoglobin the brownish colored cyanmethemoglobin is the product of almost all form of hemoglobin found in blood except HBS this is measured calorimetrically at 540 nm. The colour intensity at this wavelength is proportional to the total hemoglobin concentration.

Reagents

Reagents 1: Hemoglobin reagent

Reagents 2: Hemoglobin standard (15g/dl)

Procedure : Four tests tubes were taken and marked as Blank (B), Standard (S), Tests (T) and control (C). To the test tube which marked as test (T) and control (C) 5.0 ml of hemoglobin reagent (Reagent-1) was added. Then, 0.02 ml of blood drawn from metal exposed fish and control fish was dropped in the respective tubes. The given hemoglobin standard reagent (Reagent-2) was taken in the test tube marked as standard (S), control (C) and test (T) against Blank (B) was measured at 540 nm using spectrophotometer (Spectronic 20D+ Milton Roy, USA).

$$\text{Calculation Hemoglobin concentration (gm/dl)} = \frac{\text{Abs. of Test}}{\text{Abs. of standard}} \text{ X } 16.31$$

RBC count (Erythrocyte Count)

The total erythrocyte count was made with Neubaur's haemocytometer. The blood was drawn up to 0.5mark in the RBC pipette and mixed the thoroughly taken in red pipette and diluted 1:200 with Hayem's fluid. The first few drops were discarded and the diluted blood sample was introduced into a counting chamber. One drop of blood was loaded in haemocytometer chamber. RBC was counted and reported as 10^6/mm^3 [7].

Erythrocytes were counted by the method of Rusia and Sood (1992) using haemocytometer.

Principle: The blood specimen was diluted with mention the name of the diluting fluid which does not remove the white cells but allow red cells to be counted in a known volume of fluid. Finally, the number of cells in undiluted blood is calculated and reported as the number of red cells per cubic millimeter of whole blood.

Procedure: Blood was drawn in a clean RBC pipette up to its 0.5 mark. The tip of the pipette was wiped clean and dipped vertically into the red cell diluting fluid, which was then gently sucked up to mark 101. Then the tip of the pipette was closed

Table 1: Toxicity of cadmium and their effect on some heamatological parameters of common carp (*Cyprinus carpio*) exposed to crude leaf extract of *Abutilon indicum*.

Parameter	Treatment	Positive control (Normal fish)	Nagative control (Cadmium)	Exposure period day (32)			
				8	16	24	32
Haemoglobin 1g/dL	C	6.3±0.05	5.6±0.1	6.6±0.1	6.8±0.15	7.30±0.06	8.34±0.1
	T	5.40±0.15	6.52±1.0	6.90±0.52	7.50±0.1	8.83±0.05	8.8±0.06
WBCs 10^3cells /ml	C	4.00±1.0	3.00±1.0	4.15±1.52	5.5±0.06	6.0±0.2	6.8±0.1
	T	6.00±0.05	4.15±1.52	5.70±1.0	5.80±1.0	6.10±1.0	6.40±1.00
RBCs x10^6 cells /ml	C	5.60±1.0	4.70±1.00	5.80±1.0	5.90±1.0	6.0±1.15	6.20±2.60
	T	6.03±1.52	4.50±1.15	6.50±0.20	6.60±0.1	7.5±0.1	7.930.15

Table 2: Survival rate % of cadmium and their exposed (*Cyprinus carpio*) exposed to crude leaf extract of *Abutilon indicum*.

S.NO		Exposed period days			
	Control	8	16	24	32
Mortality rate (%)	70%	60%	55%	40%	20%

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with a finger and the contents were mixed thoroughly by shaking the pipette at right angles to its long axis. The red bead in the bulb helps for proper mixing of blood with the diluting fluid. The counting chamber of the haemocytometer was washed with distilled water, covered with a clean special cover glass and focused under a compound microscope. The ruled area of the haemocytometer was located clearly. Then the first drop of the fluid in the pipette was discarded by holding the pipette at 450 nm. The tip of the pipette was touched between the cover slip and the counting chamber and the diluted blood was applied by blowing. The blood was drawn into the chamber was left as such for 3 minutes to allow the cells to settle down.

Counting: The slide was first examined under low power and then under high power magnification. The counting chamber of the haemocytometer has a central heavy ruled area of 1 sq. mm. This central area is RBC counting chamber. It is divided into 25 squares and each square is sub-divided into 16 small squares. For the erythrocyte count, the cells falling with in and those touching the right and upper margin of the four corner squares and the central square (8.0 small squares) were counted. The total number of erythrocytes per cubic millimeter of whole blood was then calculated.

Calculation:

$$\text{Erythrocytes} = \frac{\text{No. of erythrocyte X Dilution counted}}{\text{Area counted X Depth of fluid}} (\text{million / cu.mm of blood}).$$

Dilution - 200

Area counted - 5 X 0.04 = 0.2 square mm

Depth of fluid - 0.1 mm

WBC count (LEUCOCYTES COUNT)

Leucocytes were counted by the method of Rusia and sood (1992) using haemocytometer.

Principle: Blood is diluted with acid solution which removes the red cells by haemolysis and also accentuates the nuclei of the white cells, thus the counting of the white cells become easy. Counting is done with a microscope under low power and knowing the volume of fluid examined and the dilution of the blood, the number of white cells per cubic millimeter in undiluted whole blood is calculated.

Procedure: Blood was drawn up to the 0.5 mark using a clean WBC pipette. Then the pipette was immediately kept in a watch glass containing WBC diluting fluid and it was drawn up to mark, taking care that no air bubbles included. The contents were mixed well by rotating the pipette between the palms of the hands. The white bead in the pipette helps for proper mixing of blood with the diluting fluid. The diluted blood was allowed to stand as such for 3 minutes for haemolysis of red cells to occur. Again the contents were mixed by rotating the pipette. After discarding the first few drops of diluted blood the counting chamber of the haemocytometer was charged with the fluid making sure that no air bubble were trapped between the cover slip and the chamber. The cells were allowed to settle down for a minute.

Counting: For the counting of leucocytes, the slide was examined under mention the magnification used low power magnification of microscope. The neubaur's counting chamber is divided into two counting area which are ruled. Each counting chamber is divided into a total ruled area of 9 sq.mm. The area of each square is 1 sq.mm area of the 4 corner slide was used for the counting of leucocytes. The cells falling within the four corners square were counted and the total number of leucocytes per cubic millimeter of whole blood was calculated.

Calculation

$$\text{Calculation Hemoglobin concentration (gm/dl)} = \frac{\text{Abs. of Test}}{\text{Abs. of standard}} \text{X } 16.31$$

Dilution - 20

Area counted - 4 X 1 = 4 square.mm

Depth of fluid - 0.1 mm

Result and Discussion

In this study the cumulative percentage of mortality, haemoglobin, RBC, and WBC were studied in disease induced *Cyprinus carpio* using different concentration of *Abutilum indium*. In control groups showed 70% mortality experimental groups showed 32 days an 20% of mortality an experimental groups. Similar result were observed by, reported that *Mikania cordata* leaf powder significantly increased non-specific immunity and decreased mortality in *C. catla* experimentally infected with Aphanomyces invadans. The *M. cordata* leaf powder supplemented diet showed significantly ($p<0.05$) high disease resistance against A. invadans infection when compared with control group. The highest percentage survival was recorded in 20ppm (71.06%) followed by 10ppm (60.95%) and 30ppm (49.84%) groups [8,9], reported that *A. hydrophila* (106 CFU/ml) injected fishes showed 89.47 % mortality and severe lesions and wound were noticed in the infected portions. The injured tails appeared reddish in colour and loss of skin layer was observed. Study the survival rate was decreased from70% to 20% for the zinc after exposing *C. batrachus* to sub-lethal concentrations of each of both metals for 15 days. Similar results were reported by other studies, on *Cyprinus carpio* [10,11], on *C. carassius* and [12], when exposed *Labeo rohita* to different concentrations of chromium. Haemoglobin content on disease induced Common carp *Cyprinus carpio* fed with *Abtilium indium* formulated diet were studied in different days of treatment (0 day to 32 day). The positive control fishes showed low level of haemoglobin content (6.3±005.g/dL) when compared to negative control fish (5.6±0.1g/dL). Different concentrations of plant extract formulated diet treated fishes showed gradual increase in haemoglobin content after different days of treatment. Let it read haematological parameter in fish can significantly change in response to chemical stressors. However, their alterations are nonspecific to a wide range of substance. In recent years haematological parameters have been used more to assess the effect of sub lethal concentrations of pollutants [13]. The RBCs count in the control groups was found to be 5.60±1.0cells/ml. The plant extract treated fishes showed the RBCs 6.03±1.52cells /ml The RBCs count was increased with increasing concentration of

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plant extract formulated diet in different day of treatment (8, 16, 24 and 32) reported that the swelling of the red blood cells (erythrocytes) may be due to an increase in protein and carbon dioxide in the blood. Thus increasing or decreasing numbers of white blood cells are a normal reaction to a chemical such as zinc and cadmium [14], demonstrating the effect of the immune system under toxic conditions. The present findings is in consistent with that of [15], who have observed increase in mean RBCs counts in the fish (*Hopliasmalabaricus)* exposed to methyl mercury. In the present study the WBCs count was varied from both experimental and control fishes. The WBCs count in the control fishes showed $4.00\pm1.0\times10^{3}$ cells /ml and the plant extract 5 formulated diet treated fishes showed maximum number of WBCs was observed. In 32 plant extract formulated diet found to be $6.8\pm0.1\times10^{3}$ cells /ml in the (32 day). Observed by increased WBC counts in *Oreochromis aureus* after mercury exposure [16], also reported the decrease level of RBC in the fresh water fish *Labeo rohita* after exposure to mixture of heavy metals. All these reports are in agreement with the present study [17], stated that increase in WBC counts in the wild *C. gariepinus* might be a protective response to stress. The increase in WBC of fish was suggested to indicate alteration in defense mechanism against the action of the highly toxic and the bioaccumulated heavy metals in fish tissues as previously. This increase in WBC count may be as a result of the prevention of damage caused by zinc in the gill, kidney, and liver tissues [18].

Acknowledgement

The authors thanks the Management, Principal and Head of the Department of Zoology, Ayya Nadar Janaki Ammal College, Sivakasi for providing facilities to carry out this research work.

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